Fascin, an actin-bundling protein expression in cervical neoplasms

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Summary

Purpose of investigation: Our objectives were (1) to examine expression of fascin in cervical tissues with chronic inflammation, intraepithelial neoplasms and invasive carcinomas, and (2) to investigate the role of fascin on endothelial migration and angiogenesis in cervical neoplasms.

Methods: In this study we investigated by means of immunohistochemistry fascin expression in 92 cervical biopsy samples representative of chronic inflammation (n=13), squamous intraepithelial lesions (SILs, n = 33) and invasive carcinomas (n = 46).

Results: Various degrees of fascin expression were observed in 94% of the samples of SILs, in 67% of the samples of invasive cervical carcinoma and in 69% of the samples of chronic inflammation. Total epithelial fascin scores of samples were significantly higher in high-grade (H)SILs compared to low-grade (L)SILs, invasive carcinoma and chronic inflammation of the cervix ($p < 0.05$). Mean microvessel count was 55.00 ± 5.17 in HSILs, 40.76 ± 3.57 in LSILs, 37.11 ± 2.91 in carcinoma and 25.69 ± 3.98 in chronic inflammation. We found a significantly higher microvessel count in HSILs compared to invasive carcinoma and chronic inflammation (respectively, p = .004, p = .000).

Conclusion: Epithelial fascin expression up-regulated when the malignant tumor cell phenotype had occurred in the cervix. Similarly, microvessel count increased with the beginning of cervical tumorigenesis.

Key words: Fascin; Cervical neoplasms; Angiogenesis.

Introduction

In many cells, the bundling of actin filaments is controlled by several different actin binding and bundling proteins. One actin bundling protein, fascin, crosslinks actin filaments via two discrete F-actin-binding sites. The actin cytoskeleton is directly involved in cell locomotion, cytokinesis, cell-cell and cell-matrix interactions, vesicular and organelle transport and the establishment and maintenance of cell morphology [1-5]. β-catenin, which is the cytoplasmic partner of E-cadherin, links cadherins to α-catenin and the actin cytoskeleton and mediates strong cell-cell adhesion in the adherence junction. The adherence junction lies immediately below the tight junction toward the basal side of the cell. The tight junction and adherence junction structures are highly dynamic in their function. Assembly and/or disassembly can be controlled by a variety of intracellular signals that ultimately influence cell-cell interactions in a physiologically appropriate manner [6, 7].

The first step in invasion and metastasis is the detachment of cancer cells from the primary tumor. The cell-to-cell adhesive function in tumors is suppressed, resulting in the detachment of cancer cells. This process is dependent on the function of the adherence junction. Invasive cervical carcinoma is a multistep process and generally develops from the precursor lesions [8].

Using an immunohistochemistry assay, we evaluated the tissue distribution of fascin in biopsy samples representative of chronic cervicitis, low-grade squamous intraepithelial lesions (LSILs), high-grade squamous intraepithelial lesions (HSILs) and invasive cervical carcinoma, with the aim of investigating the role of actin-bundling protein fascin in cervical tumor progression and angiogenesis.

Materials and Methods

The samples for this study were retrieved from the Pathology Department of Osmangazi University Hospital. Ninety-two cervical biopsy samples representative of chronic inflammation (n = 13), SILs (n = 33) and invasive cervical carcinomas (n = 46) were included in the study. All the biopsy samples were reexamined histopathologically by three pathologists. Local cellular immune response assessed with a three-point scale and presence or absence of inflammation and necrosis, metaplastic changes, grades of SILs, histopathologic types and grades of malignant samples, lymphatic invasion and lymph node metastasis were recorded. All malignant cervical neoplasms were clinically staged according to (FIGO) criteria. Patient characteristics and clinicopathologic findings were obtained from hospital records. Stage and histopathologic distribution of invasive carcinomas after clinical and histopathologic review are given in Table 1. Paraffin blocks of the most representative sections of invasive tumors were obtained from the study samples. The paraffin blocks were cut at 4 μm and immunohistochemical assays were performed by using liquid mouse monoclonal antihuman fascin antibody, clone IM20 (Novocastra, NCL-L-FASCIN, USA). Tissue sections were deparaffinized in xylene, rehydrated in alcohol solutions, and placed in 0.5% hydrogen peroxide in methanol for 10 min to block endogenous peroxidase activity. Rehydration was completed by placing the sections in absolute alcohol and finally in water. The slides were treated with a boiling solution of freshly prepared 0.05 M-citrate buffer, pH 6.0 for 5 min in a pressure cooker. The sections were reacted

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Table 1. — *Stage and histologic distribution of invasive carcinomas.*

Variable	Cervical carcinoma
FIGO stage	
1A1	4
1A2	2
1B1	9
1B2	7
IIA	3
IIB	6
IIIB	15
Grade	
1	11
2	28
3	1
Histologic subtype	
Minimal invasive	6
Large cell keratinizing	11
Large cell non-keratinizing	25
Small cell carcinoma	1
Adenocarcinoma	2
Mucoepidermoid carcinoma	1
Total samples	46

overnight with the primary antibodies at a dilution of 1:200 in buffer. They were rinsed in phosphate buffered saline (PBS) before being treated with biotinylated universal secondary antibody for 10 min. After further rinsing, the slides were treated with avidin-biotin-peroxidase complex (Novocastra, Novostain Universal Detection Kit) and rinsed again. Immunostaining was accomplished by incubating them with 3 amino-9-ethylacarbazole (ACE) for 7 min and the slides were then rinsed in distilled water and counter-stained with Mayer's haematoxylin. Sections of human tonsil were used as positive controls. Capillary endothelium was also used as an endogenous positive control. As a negative control, the primary antibody was replaced by PBS.

Tissue samples were evaluated in three groups according to percentage of stained cells: ≤ 10% as 1; < 11-50% as 2; 51-100% as 3. The intensity of immunostaining was scored on a three point scale: 1 = weak; 2 = moderate; 3 = intense. A weighed score for each tumor specimen was produced by the sum of the percentage score with the intensity score and was defined as 'total epithelial fascin score'. The intensity of staining of fibroblasts and antigen presenting dendritic cells in the subepithelial layer or stromal tissue was also assessed as weak, moderate and intense. The role of fascin in endothelial cell migration and angiogenesis were investigated for each case by counting microvessels in subepithelial/peritumoral or intratumoral areas with the highest vascularity. The microvessel count was performed with 15 centimeter monitor of a digital camera (Nikon Digital Sight, DS-5M-L1) on each area at a X200 field (X20 objective and X10 ocular). The frame area for each captured image was the same. Each tumoral area was selected in the peripheral portion of the tumor including approximately equal portions of tumor and stroma for each tumor. The homogeneous or heterogenous staining pattern of fascin expression in microvessels were assessed both in the non-neoplastic and neoplastic group.

All statistical analyses were performed using SPSS (Statistical Package of Social Sciences, Chicago, IL, USA) for Windows version 11.5. Data were analyzed according to the Mann-Whitney test, Kruskal-Wallis test, t-test, Fisher's exact chi-square test, continuity correction, and ANOVA. Multiple comparison procedures were performed using Sigma Stat; statistical significance was established at $p < 0.05$.

Results

Mean ages of patients with chronic inflammation of cervix, intraepithelial neoplasms and invasive carcinoma were 47, 42 and 46, respectively. Among the invasive carcinomas, 25 patients had no parametrial involvement (Stage I-IIa) and 21 patients had parametrial involvement (Stage IIB-III) according to FIGO clinical staging criteria.

Chronic cervicitis

Fascin staining in ectocervical epithelium was marked mainly in the basal and parabasal cells. The intensity of this staining pattern decreased progressively from moderate to weak, moving from the basal layer to the spinous layer. There was no staining in the endocervical epithelium (Figure 1). Nine out of 13 samples (69%) stained with fascin and the total epithelial fascin scores ranged from zero to four. A few dendritic cells and fibroblasts stained with fascin in the subepithelial portion of 11 out of 13 samples (84%). Microvessel endothelium stained homogeneously in seven samples (53%). Mean microvessel count was 25.69 ± 3.98.

Squamous intraepithelial lesions

Fascin staining in LSILs were marked mainly in the basal and parabasal portion of the ectocervical epithelium. Metaplastic epithelium also stained with fascin. The intensity of this staining pattern decreased progressively from moderate to weak, moving from the basal layer to upper layers (Figure 2). The ten out of 11 samples (91%) stained with fascin and the total epithelial fascin scores ranged from 0 to 4. A few dendritic cells were stained in one case in the subepithelial portion. Microvessel endothelium stained homogeneously in three samples (27%). Mean microvessel count was 40.76 ± 3.57. In HSILs, regional homogeneous or heterogenous labeling of malignant keratinocytes was found (Figures 3 and 4). The 21 out of 22 samples (95%) stained with fascin and the total epithelial fascin scores ranged from 0 to 5. Inflammatory host responses contained fascin positive dendritic cells and weakly stained fibroblasts were seen in 14 out of 22 samples (63%). Microvessel endothelium stained homogeneously in five (22%) samples. Mean microvessel count was 55 ± 5.17.

Invasive cervical carcinoma

Diffuse or regional staining with homogeneous or heterogenous intensity ranging from weak to intense was observed in samples with squamous cell carcinoma (Figure 5). Horn pearls of squamous cell carcinomas, areas exhibiting acantolysis and solitary dyskeratotic keratinocytes were negative for fascin. One case of mucoepidermoid carcinoma and one case of adenocarcinoma stained in focal areas exhibiting an epidermoid pattern and squamous carcinoma in situ. Comparison of samples with invasive cervical carcinomas, SILs and chronic cervicitis is given in Table 2. Overall, fascin immunoreac-

Fig. 1

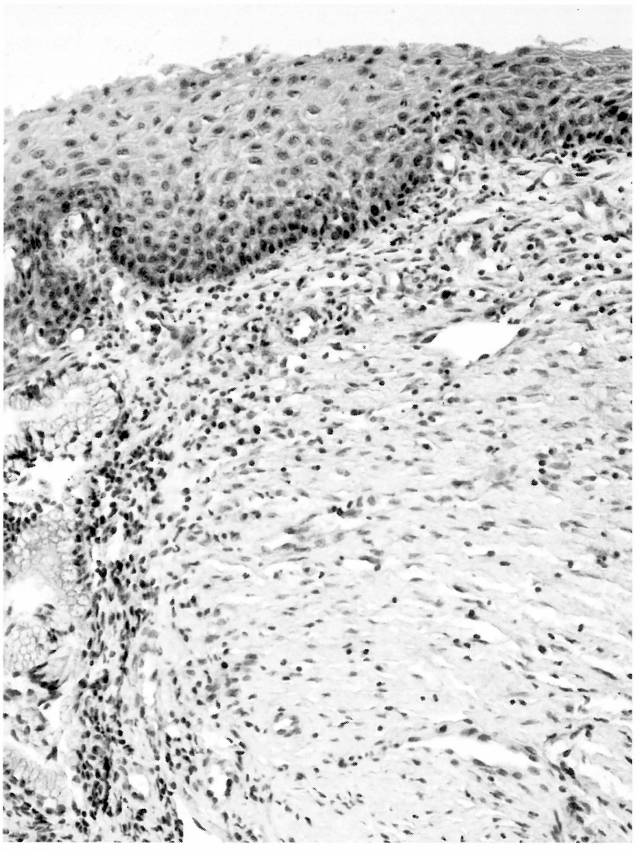

Fig.

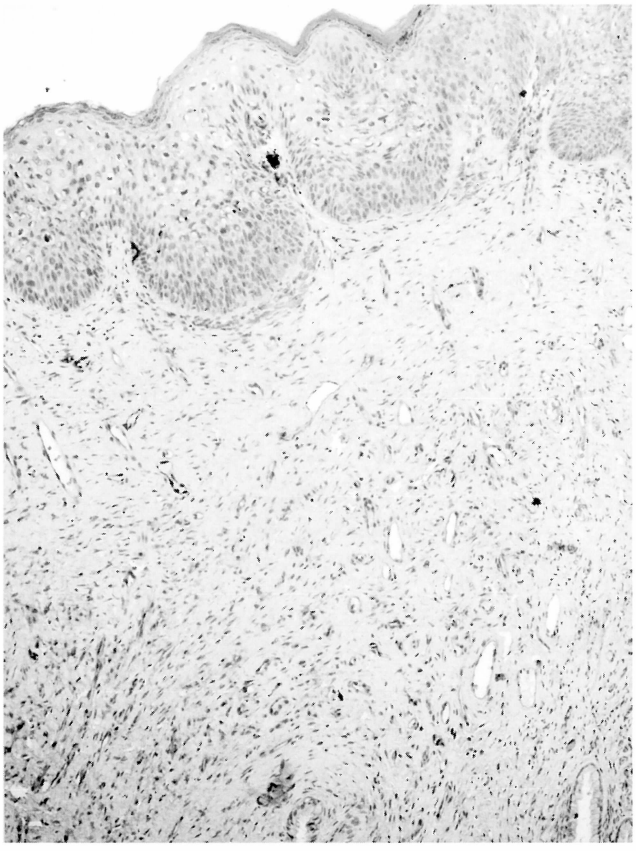

Fig. 3

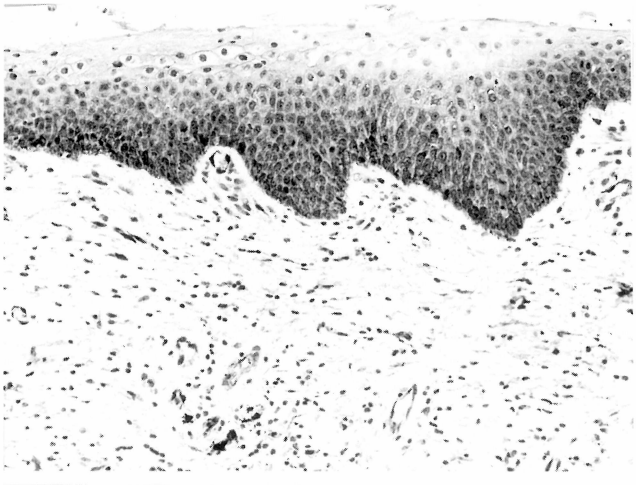

Fig.

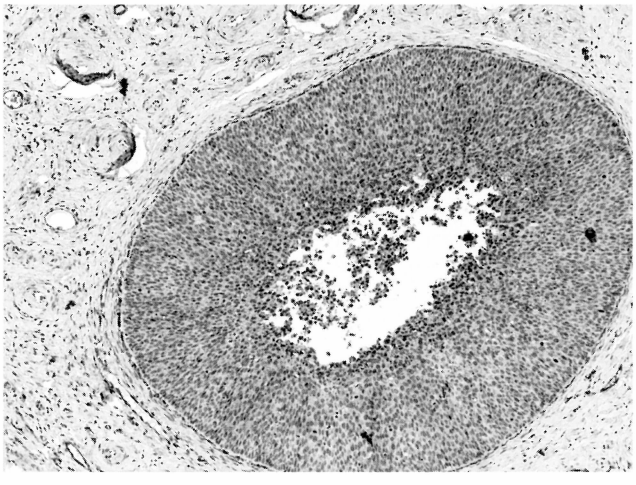

Fig. 5

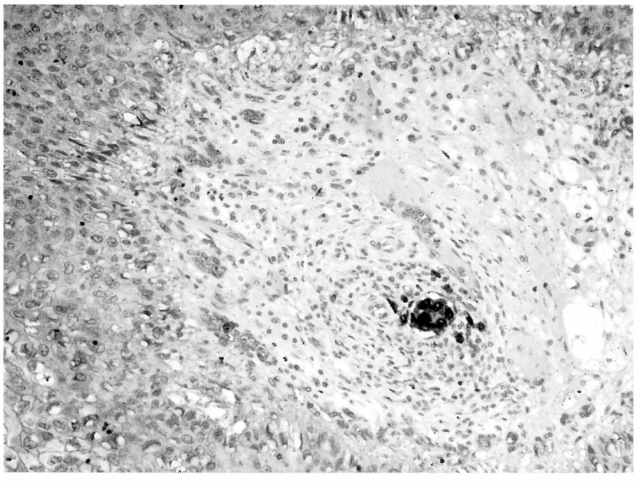

Figure 1. — Chronic cervicitis - basal portion of epithelium moderately stained for fascin (x 400).

Figure 2. — LSIL - basal portion of metaplastic cervical epithelium weakly stained for fascin (x 400).

Figure 3. — HSIL - homogeneous and moderate degree of fascin expression in the lower two-thirds of the ectocervical epithelium (x 400)

Figure 4. — HSIL - metaplastic glandular epithelium showing weak and heterogenous fascin expression (x 400).

Figure 5. — Lymphatic invasion in invasive carcinoma. Tumor cells in the microvessel lumen stained more intensely for fascin than those of tumoral tissue (x 400).

Table 2. — *Comparison of samples with invasive cervical carcinomas and SILs with chronic cervicitis.*

		Variable		p	Test
Total epithelial fascin score (0-6)		n	Mean rank	.009*	Kruskal-Wallis Multiple comparison with Dunn's method
	1. Cervicitis	13	45.00		Difference between 3 vs 4, $p < 0.05$*
	2. LSIL	11	52.04		Difference between 3 vs 1, $p > 0.05$ and 2 vs 4, $p > 0.05$
	3. HSIL	22	62.05		
	4. Carcinoma	46	39.27		
Fascin staining of microvessels		Homogeneous	Heterogenous	.292	Pearson exact chi square
	Cervicitis	7	6		
	LSIL	3	8		
	HSIL	5	17		
	Carcinoma	16	30		
Mean microvessel count	Neoplastic**	Cervicitis		.007*	t-test
	42.21 ± 2.35	25.69 ± 3.98			

* Statistically significant; ** SILs and invasive carcinomas are included in same group for comparison.

Table 3. — *Comparison of mean microvessel counts.*

Variable	N	Mean	Standard error	Minimum	Maximum	Test
Chronic cevicitis	13	25.69	3.98	5	53	
LSIL	13	40.76	3.57	20	56	
HSIL	19	55.00	5.17	21	105	
Carcinoma	44	37.11	2.91	0	113	
Total	89	39.79	2.17	0	113	.000* ANOVA

*Statistically significant

Table 4. — *Comparison of the mean microvessel counts with Post Hoc Tests (Tukey HSD).*

Variable		Mean difference	Standard error	Significance
Cervicitis	LSIL	-15.07	7.33	.176
	HSIL	-29.30*	6.73	.000*
	Carcinoma	-11.42	5.90	.222
LSIL	Cervicitis			
	HSIL			
	Carcinoma	15.07	7.33	.176
	HSIL	-14.23	6.73	.157
	Carcinoma	3.65	5.90	.926
HSIL	Cervicitis	29.30*	6.73	.000*
	LSIL	14.23	6.73	.157
	Carcinoma	17.88*	5.13	.004
Carcinoma	Cervicitis	11.42	5.90	.222
	LSIL	-3.65	5.90	.926
	HSIL	-17.88*	5.13	.004*

* Statistically significant

tivity was detected in 31 out of 46 (67%) invasive cervical carcinomas. The total epithelial fascin scores ranged from 0 to 6. Inflammatory host responses containing fascin positive dendritic cells and fibroblasts were seen in all samples ranging from weak to intense. Microvessel endothelium stained homogeneously in 16 (34%) samples. Mean microvessel count was 37.11 ± 2.91. Lymphatic invasion was detected in 38 (82%) samples. Neoplastic emboli were decorated by antifascin antibody ranging from weak to intense in 32 (84%) samples. Nodal metastasis was observed in 14 (30%) patients. Tumor cells showed heterogenous fascin staining in one lymph node. Only dendritic cells were stained with fascin in the other metastatic lymph nodes. There were significant differences among invasive carcinomas, SILs and chronic inflammation in terms of total epithelial fascin scores ($p = .009$). Multiple comparison procedures revealed that a statistically significant difference was observed between HSILs and carcinoma samples ($p < 0.05$). Mean microvessel count was 42.21 ± 2.35 in the neoplastic group and 25.69 ± 3.98 in tissues with chronic inflammation. This difference was statistically significant ($p = .007$). Comparison of mean microvessel counts among groups is given in Tables 3 and 4. There were significant differences among invasive carcinomas, LSILs, HSILs and cervicitis in terms of mean microvessel count with ANOVA (.000). Multiple comparison procedures revealed that HSILs were different from invasive carcinomas and chronic inflammation of the cervix, respectively $p = .004$ and $p = .000$. There were no significant differences between chronic inflammation of the cervix, LSILs, HSILs and invasive carcinomas in terms of homogeneous or heterogenous fascin staining of microvessel endothelium ($p = .292$, $p > 0.05$). Comparison of clinical and pathologic findings and total epithelial fascin scores in cervical carcinomas is given in Table 5. There was no significant difference in stromal staining with fascin among the neoplastic and nonneoplastic groups ($p = .121$, $p > 0.05$). There was no significant difference in the total epithelial fascin score and the presence or absence of lymph node metastasis ($p = .711$, $p > 0.05$), lymphatic invasion ($p = .087$, $p > 0.05$) and local cellular immune response ($p = 1.000$, $p > 0.05$) in invasive cervical carcinomas. There was no significant difference in total epithelial fascin scores and grade of neoplasms ($p = .403$, $p > 0.05$) and stage of tumors ($p = .801$, $p > 0.05$).

Table 5. — *Comparison of clinical and histopathologic findings and total epithelial fascin scores in cervical carcinoma of the uterine cervix.*

Total epithelial fascin score (0-6)	Variable			p	Test
Grade	Grade	n	Mean rank	.403	Mann-Whitney U
	1*	17	20.97		
	2	28	24.23		
Pelvic paraaortic lymph nodes	Absence	32	23.03	.711	Mann-Whitney U
	Presence	14	24.57		
Lymphovascular space involvement	Absence	8	16.38	.087	Mann-Whitney U
	Presence	38	25.00		
Fascin staining of tumor cells in vascular spaces (±)	Absence	5	16.40	.194	Mann-Whitney U
	Presence	41	24.37		
Stage	≤ IIA	25	23.08	.801	Mann-Whitney U
	≤ IIB	21	24.05		

* Microinvasive carcinoma cases included in the grade 1 carcinoma group for comparison.

Discussion

Fascin is expressed at high levels in specialized normal cells such as neural cells, antigen-presenting dendritic cells, endothelial cells, squamous epithelium and many transformed cells. Fascin localizes in membrane ruffles, microspikes, stress fibers and lamellipodia and its overexpression in epithelial cells induces membrane protrusions and other motility-associated structures. Fascin containing actin bundles provide mechanical support in adherence junctions [1-4]. In normal skin, fascin can be found in basal keratinocytes, endothelium, dermal dendrocytes and melanocytes whose specific cellular morphologies may depend on fascin-actin interactions in sustaining the cytoskeleton and/ or cell-cell and cell-matrix adhesions [9].

Co-localization of fascin with β-catenin by immunofluorescence supports the interactions. Catenins mediate interactions between cadherins and the actin cytoskeleton [6, 7].

Malignant transformation is a complex multistep processs in which oncogenic cells first become tumorigenic then ultimately metastatic. Fascin overexpression has been demonstrated in several types of human neoplasms including some lymphoproliferative disorders, Hodgkin's lymphoma, breast, ovarian and pancreatic carcinomas, small and non-small cell lung carcinomas, esophageal, gastric and colonic carcinomas, and skin tumors [10-19]. Down-regulation of fascin and loss of cell-cell, cell-matrix adhesions also have an important role in malignant tumor progression in some tumors, such as melanomas [9]. The role of fascin in generating and maintaining dynamic tumorigenic phenotypes has been supported by these studies.

Wong *et al.* [20] demonstrated that glucocorticoid hormones and dexamethasone strongly and reversibly down-regulate the level of fascin and stimulate transepithelial resistance which promotes the remodeling of apical junctions in rat mammary epithelial tumor cells. Guan *et al.* [21] demonstrated that transforming growth factor-α abrogates the glucocorticoid stimulation of tight junction formation and reverses the steroid induced down-regulation of fascin in rat mammary epithelial tumor cells by a ras-dependent pathway. In the colonic carcinoma cell lines up-regulation of fascin associates with aggregation of growing cells and results in glandular differentiation as manifested by polarization of nuclei toward the basal surface of cells and the organization of cells around a central lumen [12].

Goncharuk *et al.* [9] suggested that up-regulation of fascin in skin neoplasms such as basal cell carcinoma indicates a role for fascin in cell adhesion, cell motility and local invasiveness. Whereas down-regulation or loss of actin-bundling properties of fascin in invasive neoplasms such as melanomas is probably associated with disorganization of cell-cell and cell-matrix contacts and may be a crucial step in the progression from locally invasive to widely disseminated cancers. Recent studies suggest that fascin's association with β-catenin is related to the microenvironment of the neoplasm and is not related to gene function [12, 22]. Shonukan *et al.* [23] demonstrated that intense neurotrophin expression was observed in normal tissue adjacent to brain metastases of melanoma. It was suggested that an interaction between fascin and neurotrophin provides a direct link between the nerve growth factor (NGF) signaling pathway and neurotrophin-mediated melanoma cell movement by down-regulation (dephosphorilation) of fascin. This study has demonstrated that there is a need for further studies which point out the effect of the TNF/NGF pathway on fascin regulation in various neoplasms.

Both up-regulation and down-regulation of fascin functionally related to various cell signaling pathways have a central role in the mitogenic, morphogenic and metabolic function of the tumorigenic cells [24-27]. To our knowledge, this is the first study which has investigated the role of fascin in cervical carcinogenesis. There were significant differences among invasive carcinoma of the cervix, LSILs, HSILs and chronic inflammation in terms of total epithelial fascin scores ($p = .009$). A significant difference was observed between HSILs and cervical carcinomas by using multiple comparison procedures ($p < 0.05$).

Increased mean microvessel count which was associated with the beginning of cervical tumorigenesis also supported the role of fascin in tumorigenesis and is compatible with the literature up to date. Recent studies [28, 29] and a study from our institution demonstrated the prognostic significance of angiogenesis in preinvasive and invasive cervical neoplasms. In this study, factor VIII related antigen was used as a marker [30]. We preferred fascin immunohistochemistry to investigate angiogenesis in order to observe the indirect effect of other cytokins on microvessels which are normally homogeneously stained by fascin. Mean microvessel count was 25.69 ± 3.98 in tissues with chronic inflammation and 42.21 ± 2.35 in the neoplastic group. We found a significantly higher microvessel count in invasive carcinoma of the cervix and SILs compared to chronic inflammation of the cervix

(.007). Down-regulation of fascin in microvessel endothelium (heterogenous fascin staining of microvessel endothelium) may enhance microcirculatory disturbances in tumor and peritumoral tissue and may have an important role in tumor progression.

Fascin is first up-regulated in LSILs and HSILs and then partially down-regulated in carcinoma in situ and microinvasive carcinoma. On the other hand, in invasive tumors and lymph node metastasis either homogeneous or heterogeneous fascin expression was observed. These findings support the dynamic role of fascin in every stage of cervical neoplasms. Further studies are necessary to reveal other regulatory molecular mechanisms of fascin in cervical neoplasms.

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References

[1] Adams J.C., Clelland J.D., Collet G.D.M. *et al.*: "Cell-matrix adhesions differentially regulate fascin phosphorylation". *Mol. Biol. Cell.*, 1999, *10*, 4177.

[2] Cohan C.S., Welnhofer E.A., Zhao L. *et al.*: "Role of the actin bundling protein fascin in growth cone morphogenesis: localization in filopodia and lamellipodia". *Cell. Motil. Cytoskeleton.*, 2001, *48,* 109.

[3] Duh F.M., Latif F., Weng Y.: "cDNA cloning and expression of the human homolog of the sea urchin fascin and Drosophila signed genes which actin-bundling protein". *DNA. Cell. Biol.,* 1994, *13,* 821.

[4] Ishikawa R., Yamashiro S., Kohama K. *et al.*: "Regulation of actin binding and actin bundling activities of fascin by caldesmon coupled with tropomyosin". *J. Biol. Chem.*, 1998, *273,* 26991.

[5] Puius Y.A., Mahoney N.M., Almo S.C.: "The modular structure of actin regulatory proteins". *Curr. Opin. Cell. Biol.,* 1998, *10*, 23.

[6] Carico E., Atlante M., Bucci B. *et al.*: "E-cadherin and α-catenin expression during tumor progression of cervical carcinoma". *Gynecol. Oncol.*, 2001, *80,* 156.

[7] Fujimoto J., Ichigo S., Hirose R. *et al.*: "Expression of E-cadherin and α- and β- catenin mRNAs in uterine cervical cancers". *Tumor. Biol.,* 1997, *18,* 206.

[8] Bristow R.E., Cervical Cancer. In: Scott J.R., Gibbs R.S., Karlan B.Y., Haney A.F. (eds.), Danforth's Obstetrics and Gynecology. 9th ed,. Philadelphia: Lippincott Williams &Wilkins, 2003, 923.

[9] Goncharuk V.N., Ross J.S., Carlson J.A.: "Actin-binding protein fascin expression in skin neoplasia". *J. Cutan. Pathol.,* 2002, *29*, 430.

[10] Grothey A., Hashizume R., Ji H. *et al.*: "C-erbB-2/Her-2 upregulates fascin, an actin-bundling protein associated with cell motility, in human breast cancer cell lines". *Oncogene,* 2000, *19,* 4864.

[11] Guvakova M.A., Boettiger D., Adams J.C.: "Induction of fascin spikes in breast cancer cells by activation of the insulin-like growth factor-1 receptor". *Int. J. Biochem. Cell. Biol.,* 2002, *34,* 685.

[12] Jawhari A.U., Buda A., Jenkins M. *et al.*: "Fascin, an actin bundling protein, modulates colonic epithelial cell invasiveness and differentiation in vitro". *AJP.,* 2003, *162*, 69.

[13] Pelosi G., Pasini F., Sonzogni A. *et al.*: "Prognostic implications of neuroendocrine differentiation and hormone production in patients with Stage I non small cell lung carcinoma". *Cancer,* 2003, *97,* 2487.

[14] Pelosi G., Barisella M., Pasini F. *et al.*: "CD117 immunoreactivity in stage I adenocarcinoma and squamous cell carcinoma of the lung: relevance to prognosis in a subset of adenocarcinoma patients". *Mod. Pathol.*, 2004, *17,* 711.

[15] Hu W., McCrea P.D., Deavers M. *et al.*: "Increased expression of fascin, motility associated protein, in cell cultures derived from ovarian cancer and in borderline and carcinomatous ovarian tumors". *Clin. Exp. Metastasis*, 2000, *18,* 83.

[16] Maitra A., Iacobuzio-Donahue C., Rahman A. *et al.*: "Immunohistochemical validation of a novel epithelial and a novel stroma marker of pancreatic ductal adenocarcinoma identified by global express microarrays: sea urchin fascin homolog and heat shock protein 47". *Am. J. Clin. Pathol.*, 2002, *118,* 52.

[17] Rong J., Xu L.Y., Cai W.J. *et al.*: "Expression of fascin 1 gene in the process of the immortalized esophageal carcinoma carcinogenesis". *Ai. Zheng.,* 2004, *23,* 243.

[18] Pinkus G.S., Pinkus J.L., Lanhoff E. *et al.*: "Fascin, a sensitive new marker for Reed-Sternberg cells of Hodgkin's disease. Evidence for a dendritic or B cell derivation?" *Am. J. Pathol.,* 1997, *150*, 543.

[19] Kempf W., Levi E., Kamarashev J. *et al.*: "Fascin expression in CD30-positive cutaneous lymphoproliferative disorders". *J. Cutan. Pathol.,* 2002, *29,* 295.

[20] Wong V., Ching D., McCrea P.D., Firestone G.L.: "Glucocorticoid down-regulation of fascin protein expression is required for steroid-induced formation of tight junctions and cell-cell interactions in rat mammary epithelial tumor cells". *J. Biol. Chem.*, 1999, *274,* 5443.

[21] Guan Y., Woo P.L., Rubenstein N.M., Firestone G.L.: "Transforming growth factor-α abrogates the glucocorticoid stimulation of tight junction formation and reverses the steroid-induced down-regulation of fascin in rat mammary epithelial tumor cells by a Ras-depentent pathway". *Exp. Cell. Res.,* 2002, *273*, 1.

[22] Krengel S., Grotelüshen F., Bartsch S., Tronnier M.: "Cadherin expression pattern in melanocytic tumors more likely depends on the melanocyte environment than on tumor cell progression". *J. Cutan. Pathol.,* 2004, *31,* 1.

[23] Shonukan O.T., Bagayogo I., McCrea P.D., Chao M., Hempstead B.: "Neurotropin-induced melanoma cell migration is mediated through actin-bundling protein fascin". *Oncogene,* 2003, *22,* 3616.

[24] Sandhu M.S., Dunger D.B., Giovannucci E.L.: "Insulin, insulin-like growth factor-1 (IGF-1), IGF binding proteins, their biologic interactions, and colorectal cancer". *J. Natl. Cancer. Inst.*, 2002, *94,* 972.

[25] Furstenberger G., Senn H.J.: "Insulin-like growth factors and cancer". *Lancet. Oncol.,* 2002, *3,* 298.

[26] Keehn C.A., Saeed S., Bickle K. *et al.*: "Expression of insulin-like growth factor-I receptor in primary cutaneous carcinomas". *J. Cutan. Pathol.*, 2004, *31*, 368.

[27] Shimabukaro K., Ichinose S., Koike R. *et al.*: "Hepatocyte growth factor/scatter factor is implicated in the mode of stromal invasion of uterine squamous cervical cancer". *Gynaecol. Oncol.*, 2001, *8,* 205.

[28] Tokumo K., Kodama J., Seki N. *et al.*: "Different angiogenic pathways in human cervical cancers". *Gynaecol. Oncol.,* 1998, *68,* 38.

[29] Obermair A., Bancher-Todesca D., Bilgi S. *et al.*: "Correlation of vascular endothelial growth factor expression and microvessel count in cervical intraepithelial neoplasia". *J. Natl. Cancer. Inst.*, 1997, *89,* 1212.

[30] Ozalp S., Yalcin O.T., Oner U. *et al.*: "Microvessel count as a factor in preinvasive and invasive cervical lesions". *Eur. J. Gynaecol. Oncol.*, 2003, *24*, 425.

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